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(54) Title: METHODS FOR MODIFYING THE T CELL REPERTOIRE OF THE IMMUNE SYSTEM

(57) Abstract

Compositions of subimmunogenic doses of substances which bind to T cell receptors on helper (TCR4) and/or suppressor cells (TCR8) are used to stabilize the immune system of an individual. The tolerogenic methods are useful in a variety of circumstances, including preventing graft rejection in transplantation, preventing and treating autoimmune diseases, inhibiting the development of acquired immunodeficiency diseases, and in desensitizing individuals to allergens. Preferred tolerogenic compounds include MHC class I and class II molecules, which may be allogeneic or xenogeneic to the individual being treated.

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METHODS FOR MODIFYING THE T CELL REPERTOIRE OF THE IMMUNE SYSTEM

Background of the Invention

The body's ability to discriminate between self and nonself becomes impaired in a variety of conditions. Such conditions include autoimmune diseases and may also include immunodeficiency diseases such as AIDS, cancer and ageing. The ability of the immune system to discriminate between self and non-self deteriorates with age, as is evidenced by an increase in the prevalence of autoimmune phenomena and cancers in aged individuals. As an individual ages, the strength of immune responses to foreign antigens decreases and the amount of autoantibodies produced during such responses to foreign antigens increases. A failure of the immune system to respond adequately to tumor tissue is a basis of the "immune surveillance" theory of oncogenesis.

While no consensus exists concerning the complex mechanisms involved in the regulation of the immune system, an idiotypic network paradigm has been developed in which the immune system is a network of cells that not only recognize foreign substances, but also recognize and regulate each other. The idiotypic network model provides a basis for understanding a wide range of immunological phenomena. It includes roles for B cells, T cells and non-specific accessory cells (A cells), antibodies, specific T cell factors and non-specific "second signal" lymphokines, and provides explanations for numerous aspects of immunoregulation. See generally, Hoffmann, Eur. J. Immunol. 5: 638-647 (1975); Hoffmann, in "Theoretical Immunology", Bell et al., (eds.) Marcel Dekker, NY, pp. 571-602 (1978); Hoffmann, in Contemp. Top. Immunobiol. 11: 185-226 (1980); Gunther and Hoffmann, J. Theoret. Biol. 94: 815-855 (1982); Hoffmann, in Regulation of

Immune Response Dynamics, DeLisi and Hiernaux (eds.), CRC Press, pp. 137-162 (1982); Hoffmann and Cooper-Willis, in Mathematical Modelling in Immunology and Medicine, Marchuk and Belykh (eds.), North Holland Press, Amsterdam pp. 31-42 (1983); Hoffmann, in "The Semiotics of Cellular Communication in the Immune System", Sercarz et al., (eds.), Springer-Verlag, NY, pp. 257-271 (1988); Hoffmann et al., in "Theoretical Immunology, Part Two", Perelson, ed., Addison Wesley, Redwood City, CA, pp. 291-319 (1988); Hoffmann and Grant, in "Mathematical and Statistical Approaches to AIDS Epidemiology," Castillo-Chavez, ed., in Lecture Notes in Biomathematics, Springer-Verlag, 83: 386-405 (1989); and Hoffmann et al., Proc. Natl. Acad. Sci. (USA) 88: 3060-3064 (1991).

The idiotypic network is most simply formulated as a two-dimensional model. Consider T cells and B cells of two specificity classes that relate to a foreign substance F, namely anti-F ("X_1 cells") and anti-anti-F ("X_2 cells"). The model is based on three kinds of symmetrical interactions between X_1 and X_2 lymphocytes, namely symmetrical stimulation, symmetrical inhibition and symmetrical killing. Symmetrical stimulation involves the cross-linking of receptors and specific inhibition is ascribed to specific T cell factors. Specific T cell factors are believed to have a molecular weight of roughly half (or less) that of an IgG antibody molecule and are therefore assumed to be monovalent. Hence free specific T cell factors are assumed to be unable to cross-link specific lymphocyte receptors. Symmetrical killing may involve antibody-dependent cellular cytotoxicity (ADCC), antibody plus complement mediated lysis and/or cytotoxic T cells (CTL). Some mechanisms involve killing that depend linearly on the concentration of the corresponding effector cells (notably cytotoxic T cells) or effector molecules (IgM plus complement), while other mechanisms have a quadratic or stronger dependence on the corresponding concentration variables (ADCC and IgG plus complement mediated killing).

A mathematical model that reflects the idiotypic network theory has been formulated (see, e.g., Gunther and Hoffmann, supra). The same variables are used for the concentrations of B cells and T cells of the same (or similar) specificities. The dynamic variables refer primarily to cell concentrations, with antibodies and specific T cell factors implicitly included in the model, that is, their effects are included without the addition of any more variables. The concentration of specific molecules secreted by cells of a particular specificity is related to the concentration of the cells that secrete the molecules, and in some cases also to the amount of stimulation that the cells receive. The concentration of antibodies is treated as being proportional to the concentration of cells that produce them. The concentration of specific T cell factors is set proportional to the product of the concentration of the cells that produce them times the concentration of cells that specifically stimulate those cells. The concentration of X_1 cells is denoted by x_1 and the concentration of X_2 cells by x_2. The two-dimensional model has the form:

$$\frac{dx_1}{dt} = S + k_1 x_1 x_2 e_1 - k_2 x_1 x_2 e_2 - k_3 x_1 (x_2)^2 e_3 - k_4 x_1$$

$$\frac{dx_2}{dt} = S + k_1 x_1 x_2 e_1 - k_2 x_1 x_2 e_2 - k_3 (x_1)^2 x_2 e_3 - k_4 x_2 \qquad (1)$$

influx; stimulation; linear killing; quadratic killing; natural death

with

$$e_q = \frac{1}{1 + \left(\frac{x_1 x_2}{C_q}\right)^{n_q}} \qquad q = 1, 2, 3$$

inhibition

The e_q terms (q = 1,2,3) are called "effectivities", and these terms model specific inhibition (inhibition of stimulation and inhibition of killing) by specific T cell

factors. The C_i (i = 1, 2, 3) are constants that specify the threshold values of the product x_1x_2 at which inhibition by specific T cell factors becomes effective, and n_1, n_2 and n_3 are constants that determine the sharpness of the thresholds for inhibition by specific T cell factors. Interactions between X_1 and X_2 cells and antibodies are assumed to be inhibitable by both idiotypic (X_1) and antiidiotypic (X_2) specific T cell factors. This is a model of the stable states of the system, and is not a model of all the events that occur during antigen-triggered induction of an immune response. Such switching involves non-specific lymphokines and accessory cells in addition to the components that are included in this model.

Suitably chosen values of the parameters lead to four stable steady states. In the virgin state there are low levels of both X_1 and X_2 clones for a given specificity, with a balance between influx of cells and a linear killing mechanism, for example IgM plus complement mediated killing. In the immune state there is an elevated level of X_1 cells and a low level of X_2 cells. In the suppressed (tolerant, unresponsive) state there are elevated levels of both X_1 and X_2 clones, and mutual stimulation between X_1 and X_2 T cells leads to inhibition by specific T cell factors. Finally, there is an "anti-immune state" which is the converse of the immune state. It has elevated X_2 and low X_1 population levels.

Trajectories in the x_1/x_2 phase plane are obtained by integrating the above pair of differential equations. As illustrated in Fig. 1, four stable states of the system, where the trajectories converge to a point, can be seen. They are labeled VS, SS, IS and AIS for Virgin State, Suppressed State, Immune State and Anti-Immune State, respectively.

The two-dimensional model has been be generalized to N dimensions by Hoffmann and his collaborators (D.J. Mathewson, M.J. Lyons and K.G. Soga) as follows. Consider clones X_i with concentrations x_i, where i has values from 1 to N. Let the strength of interaction (affinity) between the V

regions of clones i and j be K_{ij}. Let e_{qi} (q = 1, 2, 3; i = 1, N) denote the extent to which function q is inhibited by specific T cell factors with complementarity to the receptors of the i^{th} clone (q = 1 for stimulation, q = 2 for linear killing, q = 3 for quadratic killing). Interactions of the i^{th} clone with the j^{th} clone are inhibited both by factors that are complementary to the V regions of clone i and by factors that are complementary to the V regions of clone j. Two corresponding terms are needed that simulate the inhibition by blocking receptors. The term e_{qi} models the blocking of the receptors of cell i by factors that have complementarity to i, and e_{qj} models blocking of receptors of the clones j that are complementary to clone i. The differential equation then has the form:

$$\frac{dx_1}{dt} = S + k_1 x_i e_{1i} U_{1i} - k_2 x_i e_{2i} U_{2i} - k_3 x_i e_{3i} U_{3i}^2 - k_4 x_i$$

The U_{qi} (q = 1, 3) are measures of the effective fields of the i^{th} clone for stimulation and killing. They are the sum of concentrations x_j of clones j that are complementary to clone i, weighted by the affinity K_{ij} and the effectivity e_{qj}.

$$U_{qi} = \sum_{j}^{N} K_{ij}\, e_{qj}\, x_j \qquad q = 1, 2, 3$$

The effectivities e_{qi} are given by

$$e_{qi} = \frac{1}{1 + \left(\frac{x_i}{c_q}\right)^{n_q}} \quad q = 1, 2, 3; \quad i = 1, N$$

or alternatively by a step function:

$e_{qi} = 1$ if $\Psi_i < C_q$

0 if $\Psi_i > C_q$ $\quad q = 1, 2, 3; \quad i = 1,N$

Ψ_i is a measure of (proportional to) the concentration of specific T cell factor that inhibits interactions with clone i. Ψ_i is computed by summing over the entire network (k=1, N), weighting each clone k firstly by its complementarity K_{ik} to clone i, secondly by its size X_k, and thirdly by the extent Y_k that it is stimulated to secrete factor.

$$\Psi_i = \sum_{k}^{N} K_{ik} x_k Y_k$$

where Y_k is the basic field of clone k as defined by:

$$Y_k = \sum_{j=1}^{N} K_{kj} x_j$$

This model is an approximation in that one is not attempting to model inhibition of stimulation of clone k to produce factor k. This is a good approximation if the level of factors needed to specifically inhibit factor production is high relative to C_1, C_2 and C_3.

To keep the total amount of specific T cell factor within a sensible range, Ψ_i is computed by the above equation, then normalized such that the average value of the Ψ_i's is mid-way between C_2 and C_3. This global regulation aspect of the model simulates a possible mechanism for keeping the total concentration of specific T cell factors approximately

constant, and ensures that not all the clones go to the suppressed state.

Fig. 2 shows the results of a numerical experiment done with this model. A sparse 20 x 20 interaction matrix K_{ij} and initial values x_i for 20 clones were generated using a random number generator. The matrix connectance (number of non-zero K_{ij} as a fraction of the total number of K_{ij}) is 0.1 in this experiment. A set of values for the parameters of the model was chosen based on the analysis of the two-dimensional model (Gunther and Hoffmann, supra). Using the random initial values of the x_i, the 20 simultaneous equations are integrated until the system reaches a stable steady state. This experiment was done three times for the same K_{ij} matrix and three different sets of values of the initial conditions. The results of Fig. 2 show that each set of initial conditions led to a different steady state. In this model the clones are classified as being in the virgin, suppressed or immune/anti-immune state according to the value of Ψ_i relative to C_2 and C_3. If $\Psi_i < C_3$ the clone i is classified as being in the immune state or the anti-immune state, if $C_3 < \Psi_i < C_2$ it is in the virgin state, and if $C_2 < \Psi_i$ it is in the suppressed state. Clones that are strongly connected to other clones tend to be in the suppressed state, clones with an intermediate level of connectance tend to be in the virgin state while clones in the immune or anti-immune state have a low connectance. This model makes it plausible that such a system can have a large number of stable steady states, with clones that are in a suppressed state being inhibited by specific T cell factors.

The immune system normally discriminates between self and foreign antigens, in that it does not respond to immunizations with self antigens as readily as it does to immunizations with foreign antigens. Thus the immune system is naturally in a suppressed state (or other unresponsive state) for self antigens.

It is possible to cause a switch to occur between stable states, for example from the virgin state to the

suppressed state for a particular foreign antigen, or from the virgin state to the immune state for that antigen, by injecting antigen in various forms, in various doses, coupled to various other agents, with or without various adjuvants, and by various routes. The "induction of specific immunological tolerance" refers to injecting antigen in a way such that subsequent challenge with the antigen (in a way that would induce immunity in a naive animal) results in no immune response, or a response that is smaller than the response that would be observed in a naive animal. Such immunological tolerance is seen mainly at the humoral immunity or cellular immunity levels (Parish, J. Exp. Med. 134: 21-47 (1971); Bretscher, Eur. J. Immunol. 9: 311-316, 1979), or at both levels (Chase, Proc. Soc. Exp. Biol. Med. 61: 257 (1946); Battisto and Miller, Proc. Soc. Exp. Biol. Med. 111: 111 (1962); Battisto and Chase, J. Exp. Med. 121: 591 (1965); Dvorak et al., J. Immunol. 94: 966 (1965); Weigle, Int. Arch. Allergy Appl. Immunol. 29: 254 (1966).

Non-specific accessory cells, namely macrophages, monocytes and dendritic cells (A cells) are known to play an important role in immune responses. The symmetrical network theory includes a postulated role for these cells in antigen-triggered switching from the virgin state to the immune state. Antigen-specific T cell factors bind to the surface of macrophages, so in the model the A cell is assumed to have a receptor for the constant part of specific T cell factors. It is postulated that the cross-linking of that receptor by the antigen via the specific T cell factor activates the A cell to secrete a non-specific factor. This non-specific factor provides B cells with a second signal, which permits them to differentiate from a form secreting very little antibody, to a plasma cell, which secretes large amounts of antibody.

Incorporation of the A cell into the model explains firstly the helper functions of T cells, and secondly the remarkable dose-response properties of the system. The postulated "second signal" role of the A cell derived non-specific factor automatically leads to lower and upper

thresholds for the amount of T cell activity that results in switching from the virgin to the immune state. In the unperturbed system there is a mixture of T cell factors of many different specificities on the A cell surface. T cells are more sensitive to antigenic stimulation than B cells. (Mitchison, in Cell Interactions and Receptor Antibodies in Immune Responses, Makela et al., eds., Academic Press, London (1971)). If a very small amount of antigen is used, it may still suffice to stimulate some relatively high affinity antigen-specific T cell clones to proliferate and secrete antigen-specific factors, but may not suffice to cause activation of the A cell to secrete a second signal factor for B cells. The proliferation of the few high affinity clones could eventually lead to the stimulation and proliferation of the corresponding anti-idiotypic clones, and thus induce the suppressed state. This would explain the phenomenon of the induction of unresponsiveness with low doses of antigen ("low zone tolerance"), which is the simplest form of switching between stable states. Dresser, Immunology 5: 161 (1962); Mitchison, Proc. Roy. Soc. London, Ser. B. 161: 275-292 (1964).

An immune response occurs when a larger dose of the antigen more effectively arms the A cell (and/or the antigen) with antigen-specific T cell factors, leading to A cell activation by the antigen via the specific factors. The activated A cell secretes a non-specific factor, which constitutes a differentiation signal for B cells to become antibody-secreting plasma cells. Only those B cells that have been activated by the cross-linking of their receptors express the receptor for the non-specific factor.

A very high dose of antigen would stimulate too many T cells, in the sense that the available sites on both the antigen and the A cell would be saturated with specific T cell factor. The activation of the A cell is then inhibited, and the B cells do not receive the "second signal". The armed A cells would nevertheless induce low affinity T cells (that are not so effectively blocked by antigen-specific factors) to

proliferate, leading again to the suppressed state with elevated levels of antigen-specific and antiidiotypic cells. This would explain the phenomenon of the induction of unresponsiveness with doses of antigen that are higher than immunogenic doses ("high zone tolerance").

Some T lymphocytes are positively selected to recognize MHC molecules, and others are believed to be selected to be similar to ("images of") MHC molecules. (Hoffmann, 1988, supra). Helper T cells are selected on the basis of their receptors having weak complementarity to class II MHC molecules, cytotoxic T cells are selected on the basis of their receptors having weak complementarity to class I MHC, and it has been suggested that suppressor T cells are selected on the basis of their receptors having similarity to class II MHC (Hoffmann (1988), supra). There is thus an intimate relationship between the repertoire of T cells and the MHC antigens of an individual. In the context of the above immune network theory, there may be a "major axis" of shapes in the immune system consisting of self MHC molecules, anti-MHC T cell receptors and MHC-image T cell receptors.

Most graft rejection is mediated by lymphocytes from the graft recipient's immune system infiltrating the grafted tissue. The infiltrating T lymphocytes recognize MHC antigens of the donor as foreign and act through several effector mechanisms to eliminate the foreign tissue. Immunosuppressive agents such as cyclosporin A and corticosteroids inhibit the elimination of the transplanted foreign tissue, but also cause generalized immunodeficiency in the recipient, leaving the graft recipient dangerously susceptible to infection. Long term administration of immunosuppressive drugs, as is necessary with mis-matched transplants, is also associated with other clinical problems such as kidney failure, hypertension and neoplasia. As the MHC genes are highly polymorphic within the outbred human population, histocompatibility typing to find appropriate MHC matches may require screening of large numbers of potential donors. This is costly, extremely time consuming, technically difficult,

and many times appropriate matching with recipients is impossible. Many potential transplant recipients die before acceptably MHC-matched organs become available.

Some prolongation of graft persistence in the presence of histoincompatibility has been achieved experimentally (Hoffmann et al., J. Immunol. 137: 61-68, 1986) using antibodies directed against the receptors of recipient lymphocytes which recognize the MHC antigens of the donor. The specificity of this protection and the autologous origin of the antibodies, which are present in alloimmune mice, suggests a role for the idiotypic network in the regulation of allograft rejection responses. Further, interactions between lymphocytes contained within the graft ("passenger lymphocytes") and the lymphocytes of the graft reacting to the host are believed to play a role in the rejection process, since graft survival can be improved by culturing the graft tissue for a period prior to grafting (Lafferty, Science 188: 259-261 (1975)). During this culture period, the passenger lymphocytes within the graft tissue presumably die, and can no longer participate in the interactions that lead to graft rejection.

A link between particular MHC molecules and a tendency to develop autoimmune disease has been demonstrated for many autoimmune diseases (see, e.g., Todd et al., Science, 240: 1003-1009 (1988)). The probability that a given individual develops an autoimmune disease depends on the HLA alleles (MHC antigens) in the individual's genome. Since the MHC antigens profoundly influence the T cell repertoire, and since T cells play an important role in regulating the immune system, the influence of MHC molecules on the frequencies of various autoimmune pathologies is believed to involve the way in which the MHC antigen influences the T cell repertoire.

In man the DR2 allele of the class II MHC molecule HLA-DR is protective for diabetes (Barbosa et al., Tissue Antigens 14: 426 (1979) and Wolf et al., Diabetologia 24: 224 (1983)), and in a mouse model for diabetes, the NOD mouse, a particular class II MHC molecule, namely I-E, has similarly

been shown to be protective in transgenic experiments (Lund et al., Nature 345: 727 (1990); Nishimoto et al., Nature 328: 432-434 (1987); and Uehira et al., Int. Immunol. 1: 209-213 (1989)). Similar findings have been obtained in the mouse AIDS ("MAIDS") model. In this case, a class I MHC molecule is protective, namely H-2D^d (Makino et al., J. Immunol. 144: 4347-4355 (1990)), although the effect depends also on the presence of other MHC molecules. There is evidence that MHC molecules can influence the rate of progression to AIDS (Steel et al., Lancet i: 1185-1188 (1988)), consistent with the proposition that AIDS may be an autoimmune disease.

AIDS is an immunodeficiency disease that results in the development of both autoimmunity (Ziegler et al., Clin. Immunol. Immunopath. 41: 305-313 (1986); Andrieu et al., AIDS Research 2: 163-174 (1986); Grant et al., J. Immunol. 144: 1241-1250 (1990); Martinez-A, Lancet, i, 8583, 454 (1988); Shearer, Mt. Sinai J. Med. 53: 609-615 (1986); Siliciano et al., Cell 54: 561 (1988); Zarling et al., J. Immunol. 144: 2992-2998 (1990)) and cancers such as Kaposi's sarcoma and lymphomas. A unifying aspect of the occurrence of both cancer and autoimmunity in AIDS is a failure of the immune system to distinguish properly between self and non-self. The importance of autoimmunity in AIDS is illustrated by the fact that 31 similarities have been noted between AIDS and *systemic lupus erythematosus* (Kaye, Ann. Int. Med. 11: 158-167, 1989). Shearer suggested that alloimmunity may be important in AIDS pathogenesis (Shearer, N. Engl. J. Med. 308: 223-224 (1983)), while Ziegler and Stites and Andrieu et al. proposed the first autoimmunity model of AIDS pathogenesis based on network ideas (Ziegler and Stites, supra; Andrieu et al., supra). A model of AIDS pathogenesis has been developed based on autoimmunity, alloimmunity and idiotypic network interactions (Hoffmann et al., (1991), supra). This model has been called the MHC-Image-Anti-MHC-Image model of AIDS pathogenesis and involves synergy between MHC-image immunity and anti-MHC-image immunity in destabilizing the immune system. Experimental evidence supporting such a model of pathogenesis has been

obtained for the autoimmune MRL-*lpr/lpr* mouse (Kion and Hoffmann, Science 235: 1138-1140 (1991). The evidence includes the finding that anti-HIV antibodies are present in these mice. These antibodies are interpreted as being anti-MHC-image antibodies, thereby linking the existence of such antibodies to autoimmune pathology. Immunization with proteins that bind to antibodies present in alloimmune, immunogen-absorbed ("AIA") sera has been found to inhibit the development of autoimmunity in mice (as described in copending USSN 07/629,331, the disclosure of which is incorporated herein by reference).

MRL-lpr/lpr mice make both MHC-image and anti-MHC-image antibodies (Kion and Hoffmann, supra). Hence the two kinds of immunity invoked in the MHC image anti-MHC-image ("MI-anti-MI") model of AIDS pathogenesis, namely MHC image and anti MHC image are present in these autoimmune mice, and the MI-anti-MI pathogenesis model may have applicability to other autoimmune diseases besides AIDS. Autoimmunity may in general occur when B cells make antibodies with shapes that are specific for the T cell V regions along the "major axis" of shape space, namely MHC-image (specific for anti-MHC T cell V regions) and anti-MHC-image (specific for MHC-image T cell V regions). It is therefore important to devise strategies for the inhibition or suppression of such B cell clones.

Chimpanzees can be infected with HIV, but the virus does not cause AIDS in chimpanzees. It has been reported that cytotoxic T lymphocytes (CTL) exist in HIV infected humans that kill normal human T cells, while HIV infected chimpanzees do not contain CTL specific for chimpanzee T cells. (Zarling et al., J. Immunol. 144: 2992-2998 (1990)).

A variety of conditions similar to AIDS exist in animals and may have etiologies similar to that of AIDS (Salzman, ed., "Animal Models of Retrovirus Infection and their Relationship to AIDS," Academic Press, 1986).

Allergies are a common health problem in which an individual makes an immune response of the IgE class to (typically) some environmental antigen. Allergies are

typically treated clinically with a desensitization procedure, consisting of injections with increasing levels of antigen. This technique is only partially successful, and it would be very useful to have a more reliable method.

Accordingly, it would be important to be able to induce changes in the T cell repertoire to prevent and treat conditions involving, among others, a failure to adequately distinguish between self and non-self, a failure to respond adequately to foreign antigens, and a failure to accept needed grafts. Quite surprisingly, the present invention fulfills these and other related needs.

Summary of the Invention

The present invention provides methods and compositions for modifying the T cell repertoire of an individual to antigens of interest as needed. By administering subimmunogenic amounts of TCR4 and/or TCR8 binding substances to the individual, an adverse immune response may be suppressed, or a desired response may be enhanced. To optimize the suppression, tolerance or other desired modulation, subsequent additional doses of the TCR4 and/or TCR8 binding substances may be necessary. In this situation the subsequent doses are typically equal to or escalating in amount compared to a previous dose. In preferred embodiments the TCR4 and TCR8 binding substances are MHC class II and MHC class I molecules, respectively, and are alloantigens or xenogeneic antigens, depending on the source of the tissue, cells or organ to be transplanted.

In another aspect of the invention, methods and compositions are provided for inhibiting autoimmune disease by administering to a subject a subimmunogenic amount of MHC class II molecule associated with the absence of the autoimmune disease and a TCR8 binding antigen, such as a class I MHC molecule.

The MHC class I and class II antigens can be allogeneic or xenogeneic MHC molecules, and can be covalently linked to one another. If the MHC molecules are xenogeneic, they are derived from a species which does not suffer from the autoimmune disease. Although a single administration may suffice to produce the desired modulation, e.g., inhibit the development of autoimmunity, subsequent additional doses of the molecules may be necessary, given in amounts which individually are equal to or greater than a previous dose. Further, booster doses may be necessary over the course of a subject's lifetime to maintain the desired state, for example, to inhibit the development of autoimmunity.

The invention further provides methods and compositions for inhibiting immunodeficiency disease in a subject, which comprises administering to a subject susceptible to developing the disease a subimmunogenic amount of one or more MHC antigens and a physiologically acceptable carrier. In this method additional doses of MHC antigens may be given in amounts which individually are equal to or greater than a previous dose, where said subsequent doses may reach or exceed doses which, if given initially to an immunologically naive subject, would cause a humoral immune response. The MHC antigens which are given are typically alloantigens of class I and/or class II, or are obtained from a xenogeneic species which is not prone to developing the immunodeficiency disease. The MHC antigens can be covalently linked to each other for optimal activity.

In related embodiments the invention also provides methods and compositions for inducing immunological tolerance to a particular antigen, as is important in the treatment of allergies. Tolerance to the antigen (allergen) comprises administering subimmunogenic amount(s) of the antigen and one or more MHC molecules. Typically the antigen and the MHC molecule will be covalently linked. As in related aspects of the invention mentioned above, additional doses of the MHC-antigen composition, in amounts which individually are equal to or greater than a previous dose, can be used to induce or

maintain tolerance to the antigen. The MHC molecule is selected from class I, class II, or a combination of MHC class I and II molecules, and can be allogeneic or xenogeneic.

Another aspect of the invention comprises administration of a subimmunogenic amount (that is, sub-immunogenic for elicitation of a humoral immune response) of a subject's own MHC molecules of one class (class I or II) in combination with self or foreign MHC of the other class. The self MHC molecules can be given in a more immunogenic form than that to which the individual is usually exposed. For example, self MHC molecules can be aggregated or covalently cross-linked to each other, and class II MHC molecules can be covalently coupled to class II MHC molecules.

Brief Description of the Drawings

Fig. 1 depicts phase plane dynamics for the two-dimensional model, equation (1), with the parameter values: $k_1 = 3$; $k_2 = 10$; $k_3 = 100$; $k_4 = 1$; $S = 10$; $C_1 = 100$; $C_2 = 10$; $C_3 = 0.1$; $n_1 = n_2 = n_3 = 5$.

Fig. 2 shows representative stable steady states of an immune network consisting of twenty clones. A network of twenty clones was modelled by generating a random K_{ij} matrix of clonal interaction strengths, and the dynamics of the system was investigated using the N-dimensional mathematical model described above. In 2a, 2b and 2c, the system started in different randomly set initial conditions and the twenty-dimensional equation was integrated until a stable steady state was reached.

Fig. 3 illustrates a model of the interactions between CD4 and CD8 T cells, that are selected to have affinity for class II MHC and class I MHC respectively. In this model there is a network of idiotypic interactions between CD8 T cell V regions and CD4 T cell V regions.

Fig. 4 illustrates a method for inducing long-lasting changes in the T cell repertoire using two substances,

one of which binds mainly to the T cell receptors of CD4 T cells ("TCR4"), and the other of which binds mainly to the T cell receptors of CD8 T cells ("TCR8"). These substances can be, for example, and as shown here, class II MHC and class I MHC respectively.

Description of the Specific Embodiments

The present invention provides a means for inducing tolerance to a variety of antigens. This is useful in a variety of circumstances, including the induction of transplantation tolerance to permit allografts or xenografts, the prevention of acquired immune deficiency syndrome (AIDS) that is associated with HIV infection in humans, treatment and prevention of autoimmune diseases and other conditions associated with the dysfunction of the immune system, such as allergies.

In one aspect the invention relates to the induction of tolerance by the administration of low doses of compounds which binds preferentially to T cell receptors on a subset of CD4 cells ("TCR4"), as exemplified by MHC class II molecules, or to T cell receptors on a subset of CD8 cells ("TCR8"), as exemplified by MHC class I molecules. The TCR4 and/or TCR8 binding substances are administered to an individual susceptible to or suffering from a disease that is treatable or preventable by achieving a suppressed state as to particular antigen(s), such as autoimmune diseases, allergies, transplantation of allogeneic or xenogeneic tissues, etc.

The molecules encoded by the MHC have been extensively studied in both the human and murine systems. In general, they have been classified as class I glycoproteins, found on the surfaces of all cells and primarily recognized by TCR8, and Class II which are found on the surfaces of several cells, including accessory cells such as macrophages, and are involved in presentation of antigens to helper T cells via

binding to CDR4. Many of the histocompatibility proteins have been isolated and characterized. For a general review of MHC glycoprotein structure and function, see Fundamental Immunology, 2d Ed., W.E. Paul, ed., Ravens Press N.Y. 1989, which is incorporated herein by reference.

The class I MHC in humans is located on chromosome 6 and has three loci, HLA-, HLA-B, and HLA-C. The first two loci have a large number of alleles encoding alloantigens. These consist of a 44 Kd heavy chain subunit and a 12 Kd β_2-microglobulin subunit which is common to all antigenic specificities. Isolation of these detergent-soluble HLA antigens has been described by, e.g., Springer et al., Proc. Natl. Acad. Sci. USA 73: 2481-2485 (1976), and Clementson et al., in "Membrane Proteins: A Laboratory Manual," A. Azzi et al., ed., 1986, pp. 57-64, which are incorporated herein by reference. The MHC glycoproteins can be isolated from appropriate cells or can be recombinantly produced. Methods for purifying the murine I-A (Class II) histocompatibility proteins are well known and have been disclosed by Turkewitz et al., Molecular Immunology (1983) 20:1139-1147, which is incorporated herein by reference.

Cloning of the MHC genes (e.g., as described by Estess et al., infra, or Steinmetz et al., Nature 300: 35-42 (1982), incorporated herein by reference) permits ready manipulation of the MHC fragments and subunits, as desired. For example, the α and β chains of seven allelic variants of the I-A region have been cloned and sequenced (Estess et al., in "Regulation of Immune Gene Expression," Feldman et al., eds., The Humana Press, Inc., 1985, pp. 3-19).

The term "isolated MHC subunit component" as used herein refers to an MHC glycoprotein subunit (i.e., an α or β chain of MHC II or a heavy chain of MHC I), which is in other than its native state, for example, not associated with the cell membrane of a cell that normally expresses MHC. This term embraces a full length subunit chain, as well as an effective portion of the MHC subunit.

Thus, by MHC molecule or fragment thereof is meant a histocompatibility molecule that is recognized as foreign by the recipient host. This includes entire histocompatibility molecules harvested from allogeneic or xenogeneic cells, as the case may be, or fragments thereof sufficient to cause an antigen-specific modification of the immune system, prepared by digestion of entire molecules, expression of recombinant MHC polypeptides as generally described in Paul et al., supra, or synthetic MHC peptide molecules of 8 to 35 amino acids, more typically 9 to 20 amino acids. An MHC fragment will typically comprise an antigen binding site and sequences necessary for recognition by the appropriate T cell receptor.

MHC proteins suitable for use in the present invention have been isolated from a multiplicity of cells using a variety of techniques, including solubilization by treatment with papain, by treatment with 3M KCl, and by treatment with detergent. In a preferred method detergent extraction of Class II protein from lymphocytes followed by affinity purification is used. Detergent can then be removed by dialysis or selective binding beads. The molecules can be obtained by isolation from any MHC bearing cell, such as B lymphocytes, e.g., from an individual or species naturally tolerant to the particular disease or condition being treated.

Isolation of individual fragments from the isolated MHC proteins is easily achieved using standard techniques known to those skilled in the art. For instance, in the case of Class I molecules, the heavy chain can be separated using SDS/PAGE and electroelution of the heavy chain from the gel (see, e.g., Dornmair et al., Cold Spring Harbor Symp. Quant. Biol. 54:409-416 (1989), and Hunkapiller et al., Meths. Enzymol. 91: 227-236 (1983), which are incorporated herein by reference). Separate α and β subunits from MHC II molecules are also isolated using SDS/PAGE followed by electroelution (Gorga et al. J. Biol. Chem. 262: 6087-6094 (1987) and Dornmair et al., supra). A number of other well known methods of separating molecules can be used, such as ion exchange

chromatography, size exclusion chromatography, affinity chromatography, or the like.

The MHC molecules and fragments can also be readily modified and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art. The amino acid sequences of a number of Class II MHC proteins are known, and the genes have been cloned, therefore, the proteins and polypeptide fragments can be made using recombinant methods. The synthetic gene encoding the MHC molecule will typically include restriction sites to aid insertion into expression vectors and manipulation of the gene sequence. The genes encoding the appropriate molecule or fragment thereof are then inserted into expression vectors, expressed in an appropriate procaryotic or eucaryotic host, such as E. coli, yeast, or other suitable cells, and the recombinant proteins are obtained. Construction of expression vectors and recombinant production from the appropriate DNA sequences are performed by methods known in the art. Standard techniques are used for DNA and RNA isolation, amplification, and cloning. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. These techniques and various other techniques are generally performed according to Sambrook et al., Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, which is incorporated herein by reference.

The recombinant techniques allow a number of modifications of the MHC molecules. For instance, recombinant techniques provide methods for carboxy terminal truncation which deletes the hydrophobic transmembrane domain. The carboxy termini can also be arbitrarily chosen to facilitate conjugation to various antigens, for example, by introducing cysteine and/or lysine residues into the molecule. The molecules can vary from the naturally occurring MHC protein sequence by amino acid substitutions, additions, deletions, and the like. In general, modifications of the genes encoding the MHC proteins or fragments may be readily accomplished by a

variety of well-known techniques, such as site-directed mutagenesis. The effect of a modification on the ability of the resulting molecule to activate T cells can be tested using standard in vitro cellular assays or the methods described in the example section, below. Amino acid sequence variants of MHC molecules can be prepared to increase the affinity of the molecule for T cell receptors, facilitating the stability, purification and preparation of the molecules, modifying plasma half life, improving therapeutic efficacy, and lessening the severity or occurrence of side effects during therapeutic or prophylactic use of MHC molecules in the present invention.

By low dose is meant an amount that is small compared to the dose typically used to induce humoral immune responses to foreign proteins, with the proviso that the initial low dose or doses may be followed by additional injections of higher doses of the same MHC antigens to reinforce the suppressed state when it has been induced or partially induced. Induced tolerance to a foreign protein in not necessarily long-lasting, hence the "stable state" is an approximation. A series of increasing doses, beginning at low doses, will most effectively and most reliably induce the suppressed state. In an "increasing dose tolerogenic immunization regimen", or simply "increasing dose regimen," the subject is exposed to a dose that induces the suppressed state, without the possibility for the immune state to be induced. By "increasing dose regimen" is defined herein as doses that begin with lower doses of the antigen than are typically used to induce immunity, and allowing enough time between injections to ensure that the immune system goes to a stable (or quasi-stable) suppressed state for the antigen, followed by occasional additional infections of the antigen as needed to ensure long-term maintenance of the suppressed state. Thus, once an immunologically unresponsive state has been established, it can be perpetuated or reinforced by subsequent periodic injections of the antigen, which can be smaller that the dose(s) used to tolerize.

An escalating dose regimen is necessitated in many cases by the fact that MHC genes can profoundly affect the immunological responsiveness for many antigens, and the dose needed to switch between the various stable (or quasi-stable) states can differ by orders of magnitude for animals with different MHC genes. Different subjects will be tolerized by different amounts of various substances, and an increasing dose regimen that will span several orders of magnitude of dose to establish tolerance will be most effective, followed by periodic boosts with small amounts of the antigen as needed to maintain the suppressed state.

Several factors play a role in determining whether the administration of an antigen is immunogenic or not. The factors include route of injection, dose, and the physical form of the antigen, namely whether or not the antigen is polymeric or contains aggregates that would be efficient in cross-linking receptors. In general, intravenous or oral administrations of antigen are non-immunogenic and favor induction of tolerance. A low dose (for example, a dose that is small compared with the doses typically used to induce humoral immune responses to proteins such as bovine serum albumin, keyhole limpet hemocyanin, tetanus toxoid or diphtheria toxoid) or repeated low doses can be tolerogenic, and low doses of antigen can induce suppressor T cells. A preferred mode of this invention is to use intravenous injections of an escalating dose of antigen, beginning with a low dose (as defined above). The initial low doses "push" the system towards the suppressed state for that antigen. Subsequent higher doses will then consolidate the suppressed state for that antigen. The MHC molecules may be administered in aggregate-free form or (particularly in the case of self MHC molecules) in an aggregated form or attached to a polymeric carrier to enhance the extent to which they cross-link receptors on T cells.

The combination of MHC class I and MHC class II molecules to induce tolerance to both classes is useful in a variety of settings, as described in more detail below.

Although offered by way of explanation and not limitation, the envisaged mechanism relates to the fact that within the idiotypic network model, an immunological perturbation has to be above a certain threshold for switching between states to occur. The stable steady states of the mathematical model have a range (or "basin") of attraction. If an antigen perturbs the system only a small amount and only transiently, the model suggests the system can then relax back to its initial state. A larger perturbation is necessary to move the system to a new stable or quasi-stable steady state. Very low doses of antigen have been shown to be capable of inducing immunological tolerance, but this normally requires that the antigen is injected repeatedly, so that there is a long-lasting perturbation to the system (Parish and Ada, Proc. Natl. Acad. Sci. 61: 556-561 (1968), and Shellam and Nossal, Immunology 14: 273 (1968)). According to the symmetric network model, and in the context of the present invention, switching from a virgin to a suppressed state for an antigen requires that there be mutual stimulation between antigen-specific and anti-idiotypic T cell clones. The model shown in Fig. 3 gives rise to the possibility that a combination of two substances can be used to perturb the T cell repertoire of an individual in a synergistic fashion, such that a firmly suppressed state is efficiently induced for both substances. The substances are chosen such that one of them binds preferentially to T cell receptors on a subset of CD4 cells ("TCR4"), while the other binds preferentially to T cell receptors on a subset of CD8 cells ("TCR8"). One could be an allogeneic class II MHC molecule, and the other an allogeneic class I MHC molecule. Fig. 4 shows an example in which a foreign class II MHC molecule is used to stimulate the CD4 clones 2, 4 and 6, while a foreign MHC class I molecule stimulates CD8 clones b, e and g. The TCR4 clone "6" is not idiotypically connected to any of the CD8 clones that are stimulated by the foreign class I MHC, so it is only transiently perturbed. The same is true for the TCR8 clone b. On the other hand, clones 2 and 4 are connected to clones g

and e respectively, which are being stimulated by the foreign class I MHC molecule. It follows that 2 and 4 are synergistically stimulated in these circumstances, and together with e and g may switch to being in the suppressed state.

In one method of the present invention, the low dose tolerance can be used to modify the T cell repertoire of a host to induce transplantation tolerance to foreign (transplant) antigens. Graft rejection can occur following replacement of inadequate organs, tissues or cells with allogeneic or xenogeneic transplants. Rejection involves the generation of an immune response against donor histocompatibility antigens expressed on the surface of cells within the transplant. The method according to the present invention involves modifying the transplant recipient's functional lymphocyte repertoire by administering, sufficiently prior to the transplantation, soluble MHC molecules or fragments thereof which are identical to or closely matched to the MHC molecules encoded by the transplant donor's histocompatibility genes. Allogeneic histocompatibility molecules (for an allograft) or xenogeneic histocompatibility molecules (for an xenograft) can be administered. In another aspect the invention includes administering low doses of histocompatibility molecules of the recipient's haplotype to the donor prior to the transplant to effectively tolerize the donor's cells to the recipient's MHC antigens.

Cells from a transplant donor can serve as a source of MHC molecules for the immunizations, assuming the donor is identified sufficiently in advance to permit the preparation of the purified low dose immunization(s). Or, a library of various MHC molecules can be developed and stored so that the particular alleles needed in a particular circumstance are available.

For preventing graft rejection, whether it be for transplanted cells, tissues or organs, the MHC antigens of the donor are preferably administered to the intended transplant

recipient, or the MHC antigens of the recipient are administered to the donor, as desired. The MHC molecules are administered in intervals, typically in multiple intravenous injections, with 1 to 10 immunizations at intervals in the range of about 3 days to 8 weeks. Typically, the MHC molecule will be given in the range of 1 ng to 100 μg per injection per kg body weight, with the preferred dosage being in the range of 10 ng to 10 μg per kg body weight. A preferred regimen will be increasing doses. For example, this can involve a series of injections beginning at an ultra low dose in the 0.1 ng/kg to 100 ng/kg range, and increasing by factors of 3 to 10, up to a maximum of 100 μg/kg given at intervals in the range of 3 days to 8 weeks. Representative tolerizing regimens for four species is given in Table 1, in which the immunization strategies combine escalating doses with very low initial doses of antigen.

Table 1. Representative increasing dose tolerogenic immunization regimens.

	Rabbit (2kg)	mouse (30g)	person (70kg)	small monkey (3kg)
Day 0	2ng	30pg	70ng	3ng
Day 28	20ng	300pg	700ng	30ng
Day 56	200ng	3ng	7μg	300ng
Day 84	2μg	30ng	70μg	3μg
Day 112	20μg	300ng	700μg	30μg

Attaining a level of tolerance sufficient to allow transplantation can readily be determined by a mixed lymphocyte reaction or induction of cytotoxic T cells. Detailed methodology for CTL assays is given by L. Hudson and F.C. Hay in "Practical Immunology", 3rd edition, Blackwell, 1989, p. 160.

Another embodiment of the invention for inducing transplantation tolerance involves injecting into a potential graft recipient an effective amount of both the foreign polymorphic class I MHC and class II MHC antigens expressed by a potential graft donor that are not expressed by the recipient. A synergistic stimulation of the subset of CD8 cells that recognizes both foreign class I MHC and the idiotypes of stimulated CD4 cells will reduce the amount of

antigen needed than if either MHC I or MHC II antigens were used alone. Moreover, the duration of the injections needed with the MHC I and II combination therapy will be shorter. If the recipient and donor have the same class II antigens but different class I antigens, another TCR-4-binding substance (e.g., a class II MHC molecule of another allele) should be injected together with donor class I MHC antigen. From one to about six injections of low dose MHC molecules are used, typically from about 1 ng up to about 1 μg or more per kg body weight of the TCR4-binding substance together with 1 ng to 1 μg per kg body weight of the TCR8-binding substance are administered at each interval, with intervals in the range of 3 days to 8 weeks. Increasing doses of the combination of the two classes of antigens can also be used.

In general, the dose will vary according to, e.g., the particular disease being treated or prevented and its severity, the particular TCR4 and/or TCR8 binding molecule and/or antigen, the manner of administration, the overall health and condition of the patient, and the judgment of the prescribing physician. An effective regimen is most simply determined by performing a mixed lymphocyte reaction (MLR) with the prospective donor cells as stimulators, and the prospective (putatively tolerized) recipient cells as responders. Pre-immunization cells (stored frozen) may be used as controls, to determine the extent of the decrease in immunological reaction. In addition to thymidine uptake in an MLR, production of lymphokines such as IL-2 in such cultures can be used as a measure of the strength of the immune activation by the stimulators, and hence (as measured by the absence or paucity thereof) the degree of tolerance that has been induced. Detailed methodology for MLR assays is given by Meo, in Immunological Methods, Lefkovits and Pernis, eds., 1: 227, and also in Practical Immunology, Hudson and Hay, 3rd ed., Blackwell, 1989, p. 160, which are incorporated herein by reference.

In a manner analogous to that with allografts, as described above, immunological tolerance to xenogeneic MHC

molecules can also be induced according to the present invention to prevent rejection of xenografts (grafts from individuals of a different species). Low doses ("sub-immunogenic," that is, doses lower than those typically required to elicit a humoral immune response to protein antigens) of the xenogeneic MHC molecule(s) to which immune tolerance is desired are administered. According to immune system network theory, such an approach works by activating both T cells that are specific for the antigens and T cells with receptors that are complementary to the receptors of lymphocytes specific for the tolerizing antigen.

While not intending to be bound by any theory, according to the immune network model an immunologically specific unresponsive state exists in which there has been positive selection of both T cells that recognize an antigen (antigen-specific T cells) and T cells that have V regions that mimic the antigen (anti-idiotypic T cells). Thus, the prevention of allograft rejection by administration of low doses of allogeneic MHC antigens can occur through changes in the T-cell repertoire of the recipient. If the T cell repertoire is selected to have anti-MHC and MHC-image specificities, then positive feedback occurs in an autocatalytic selective loop, in which antigen-specific idiotypes select anti-idiotype-bearing lymphocytes and anti-idiotypic idiotypes select idiotype-bearing lymphocytes. An increasing dose tolerogenic immunization regimen is thus able to induce a suppressed state. Allogeneic or xenogeneic class I MHC molecules will stimulate mainly CD8 clones, whereas allogeneic or xenogeneic class II MHC molecules will stimulate mainly CD4 cells. If class I and class II MHC molecules are given together, then there will be simultaneous direct stimulation of both CD4 and CD8 clones. The idiotypic connectance between CD4 and CD8 clones is sufficiently high that some of the CD4 clones that are being stimulated will be stimulated by idiotypes of some of the CD8 clones being stimulated and vice versa.

The invention further provides methods for modifying the immune system of an individual to protect the individual from autoimmune diseases for which protective alleles are identified. According to this method, a combination of MHC class I and MHC class II molecules is administered in a way that efficiently induces tolerance to both class I and class II MHC molecules. The method is designed to cause a long-lived modification of the immune system that is functionally similar to that caused by the permanent presence of a naturally-expressed protective MHC antigen.

Specific autoimmune dysfunctions are correlated with specific MHC types. Methods for identifying which alleles, and subsequently which MHC encoded polypeptides, are associated with an autoimmune disease are known in the art. A method described in Todd et al., EP 286,447 (which is incorporated herein by reference) is suitable. For example, over 30 autoimmune diseases are presently known, including myasthenia gravis (MG), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM), etc. Table 2 of the Todd et al., publication lists several HLA-associated autoimmune diseases, and is specifically incorporated herein by reference. For example, over 90% of rheumatoid arthritis patients have a haplotype of DR4(Dw4), DR4(Dw14) or DR1. With SLE, in both the human and murine forms of the disease, a strong association with MHC gene products has been shown for HLA-DR2 and HLA-DR3 individuals (Reinertsen, et al., N. Engl. J. Med (1970) 299:515), while in mice ($H\text{-}2^{d/u}$), a gene linked to the $H\text{-}2^{u}$ haplotype contributes to the development of lupus-like nephritis. Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D. IDDM populations have a high frequency of HLA-DR3 and -DR4 alleles, and the NOD mouse strain ($H\text{-}2K^{d}D^{b}$) is a murine model for autoimmune IDDM. Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system which mimics in many respects the human disease of multiple sclerosis (MS), linked to DR2. The disease can be induced in many species,

including mice and rats. Methods of inducing the disease, as well as symptomology, are reviewed in Aranson (1985) in The Autoimmune Diseases (eds. Rose and Mackay, Academic Press, Inc.) pp. 399-427, and in Acha-Orbea et al., Ann. Rev. Imm. 7:377-405 (1989), which are incorporated herein by reference.

For treatment according to the present invention, an individual having (or susceptible to) an autoimmune disease is identified, and the autoimmune dysfunction is identified. Identification may be by symptomology and/or an examination of family histories. The individual's MHC type is determined by one or more of several methods known in the art, including, for example, cell typing by MLR, by serologic assay, and by DNA analysis (including RFLP and PCR techniques). The individual is then treated with MHC class I and/or class II molecules together with administration of the autoantigen, which is able to suppress the immune response to the specific autoantigen. Therapy is monitored by observing the decrease in immune reactivity (cellular and/or humoral) to the autoantigen.

In another aspect of the invention, an individual having (or susceptible to) an autoimmune disease is treated with a sub-immunogenic amount (sub-immunogenic for elicitation of a humoral immune response) of his own class II MHC molecules, in combination with self or foreign class I MHC molecules, or of his own class I MHC molecules in combination with self or foreign class II MHC molecules. In order to perturb the system significantly, the self MHC molecules are given in a form that is more immunogenic than that to which the individual is usually exposed. The immunogenicity of the self MHC molecules can be enhanced by non-covalent or covalent aggregation. MHC molecules of a given class (class I or class II) may be covalently coupled or aggregated either to molecules of the same class or to molecules of the other class. The combinations are used to preferentially stimulate T cells with self anti-MHC and MHC-image specificity, and thus inhibit any B cells that have MHC-image or anti-MHC-image specificity. This method is intended for both prevention and

therapy of autoimmune disease. It permits the immune system of the subject to become more firmly established in a suppressed state for self MHC specificities, and thus inhibits the development of any autoimmune disease that involves the production of MHC-image and/or anti-MHC-image antibodies. The method also permits the suppressed state for self MHC antigens (and for self-MHC-image V regions) to be regained in the case of a patient that is already suffering from an autoimmune disease involving the presence of MHC-image and/or anti-MHC-image antibodies. Therapy is monitored by observing a decrease in the production of MHC-image, anti-MHC-image and other disease associated autoantibodies.

In another aspect of the invention, an increasing dose tolerogenic immunization regimen of xenogeneic MHC molecules is used to modify the T cell repertoire in a way that prevents or inhibits immunodeficiency diseases, including AIDS and other autoimmune diseases. The increasing dose tolerogenic immunization regimen involved doses similar to or slightly higher (up to about 10- to 100-fold) than those for allogeneic MHC molecules. Diseases that are particularly susceptible to this treatment are those that do not exist in the species of origin of the xenogeneic MHC molecules. For example, certain species such as the chimpanzee and rabbit can be infected with HIV but do not get AIDS. According to the idiotypic network analysis of HIV pathogenesis, this is related to differences between human and chimpanzee (or rabbit) MHC molecules.

For example, according to this method the disease AIDS can be ameliorated or development thereof inhibited by administering to an HIV infected individual, or to an individual susceptible to HIV infection, TCR4 and/or TCR8 binding substances in an increasing dose tolerogenic regimen. Among the preferred TCR4 and TCR8 binding substances in this method are MHC class I and/or class II molecules obtained from a xenogeneic species (or allogeneic individuals) not typically susceptible to developing the disease, e.g., chimpanzees. An HIV infected individual in a pre-AIDS condition can be given

an increasing dose tolerogenic regimen of MHC class I and class II molecules obtained from chimpanzee cells in a dosage regimen as described herein, and susceptibility to developing the disease is prevented or inhibited.

In another aspect the invention provides a method for inducing immunological tolerance to an antigen. This method is useful in preventing or treating allergies, for example. A tolerogenic vaccine is provided that includes, as active components, an antigen together with one or more TCR-binding substances, the preferred embodiments of the TCR-binding substances being either a TCR8-binding substance or a combination of TCR8-binding and TCR4-binding substances. Thus, to induce a suppressed state toward a primary antigen, low doses of the primary antigen are injected with a second antigen that is recognized by many T cells, such as MHC molecules, which may be either xenogeneic or, more preferably, allogeneic. The second antigen may be covalently coupled to the primary antigen. Coupling to an antigen for which an animal is already in a suppressed state will enhance the tolerogenicity of that antigen. Protein antigens can be conjugated to the MHC molecule or peptide by standard dehydration reactions using carbodiimides. Heterobifunctional linkers such as SPDP, glutaraldehyde and the like can also be used. Alternatively, the entire complex may be made directly from the appropriate encoding DNA using recombinant methods.

In another aspect, the invention provides a method for sensitizing an individual for subsequent immune response. The administration of soluble allogeneic or xenogeneic class I MHC molecules in certain doses activates the immune system in a way that results in a very substantial increase in the immune response to a subsequently administered antigen. MHC-I is thus a novel activator that is free of the side effects that typically characterize adjuvants. It has also been shown that the immune system is activated in a way such that a subsequently administered antigen evokes a strong immune response with a low ratio of IgE to IgG. It is unexpected (and counterintuitive) that xenogeneic or allogeneic MHC-I,

which stimulates CD8 cells rather than CD4 (helper) T cells, causes an enhanced humoral immune response.

Administration of the increasing dose tolerogenic regimen is typically systemic and is effected by injection, preferably intravenous, and thus formulations compatible with the injection route of administration may be used. Suitable formulations are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985), which is incorporated herein by reference. A variety of pharmaceutical compositions comprising low dose TCR4 and/or TCR8 binding substances, together with selected antigen as noted herein, and pharmaceutically effective carriers can be prepared, including incorporation into liposome formulations, e.g., as described in U.S. Patent No. 4,837,028, which is incorporated herein by reference. The pharmaceutical compositions are suitable in a variety of drug delivery systems, a brief review of which appears in Langer, Science 249: 1527-1533 (1990) which is incorporated herein by reference.

The pharmaceutical compositions are intended for parenteral, topical, oral or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration, as discussed in more detail above.

Preferably, the pharmaceutical compositions are administered intravenously. Thus, this invention provides compositions for intravenous administration which comprise a solution of the complex dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, e.g., water, buffered water, 0.4% saline, and the like. For instance, phosphate buffered saline (PBS) is particularly suitable for administration of soluble molecules of the present invention. These compositions may be sterilized by conventional, well-known sterilization techniques, or may be sterile filtered. The

resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

The compositions can be administered according to the methods of the present invention for therapeutic or prophylactic applications. In therapeutic applications, compositions are administered to a patient already suffering from a disease, as described above, in an amount sufficient to cure or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease and the weight and general state of the patient and are discussed generally above. In prophylactic applications, compositions containing the MHC or other molecules as indicated above are administered to a patient susceptible to or otherwise at risk of a particular disease. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend on the patient's state of health and weight, and doses will generally be in the ranges set forth above.

The following examples are offered by way of illustration, not by way of limitation.

EXAMPLE I

This Example illustrates the induction of tolerance to prevent graft rejection by administering sub-immunogenic amounts of class I MHC antigens and, separately, class II MHC antigens.

Soluble MHC molecules and fragments are prepared for administration to an intended graft recipient. In these experiments, to purify class II antigens from B cell lines the procedure of Jensen et al., J. Exp. Med. 174:1111, incorporated herein by reference, is used. Briefly, cells are grown in bulk (i.e., roller bottles), and 10 g of cells (approximately 10^{10} cells) are washed in HBSS and resuspended in 50 ml 25 mM Tris, pH 8.0 containing freshly added protease inhibitors (2 mM iodoacetamide, 1% (vol/vol) aprotinin, 1 mM PMSF), and incubated for 60 min. at 4°C, gently stirring with a magnetic stir bar to allow hypotonic lysis. Lysed cells are then centrifuged, 2000xg for 10 min., to remove nuclei, and then centrifuged at 100,000xg for 60 min. to pellet membranes. The membrane pellet can be stored at -70°C indefinitely at 10^9 cell equivalents/ml in 25 mM Tris, pH 8.0 containing protease inhibitors.

To purify the class II molecules from the cell membrane pellet, the membrane pellet is thawed and the volume adjusted to 20 ml with 25 mM Tris, pH 8.0, 1% NP-40 with freshly added 2 mM iodoacetamide, 1% aprotinin, and 1 mM PMSF (the sample is not allowed to warm during thawing). The sample is solubilized at 4°C overnight, then the lysate centrifuged at 2000xg for 10 min. and the pellet discarded. The supernatant is centrifuged at 100,000xg for 30 min. to clear the lysate.

Columns are set up in series with a) sepharose 4b, b) mouse IgG-protein A sepharose, c) anti-class II monoclonal antibody-protein A sepharose, d) second anti-class II monoclonal antibody. The immunoaffinity columns are prepared according to Schneider et al., J. Biol. Chem. 257:10766-10769 (1982) or Gorga, J. Biol. Chem. 262: 6087 (1987). The sample is loaded over ~4 h. Note that after the lysate is all in the first column, a reservoir containing 50 mM Tris, pH 8.0, 0.15 M NaCl, 0.5% NP-40, 0.01% azide is attached to provide buffer to chase the original lysate thoroughly through the columns. About 100-150 ml buffer is run through the columns. Columns are dismantled, the pre-clearing columns are washed briefly

and stored in 0.5% NP-40 buffer with azide. The mAb columns are individually washed overnight with ~100 column volumes of 50 mM Tris, pH 8.0, 0.5 M NaCl, 0.5% NP-40, 0.01% azide. For the detergent exchange, wash with ~50 column volumes of 50 mM Tris, pH 8.0, and rinse down the sides of the glass column to remove all NP-40. About 5-10 volumes of 50 mM Tris, pH 8.0, containing 1% N-octylglucoside are dripped through slowly over ~1 h. to allow detergent exchange.

The MHC molecules are eluted from the column with 5-6 volumes of 50 mM glycine-NaOH, pH 11.0, 0.15 M NaCl. Fractions are collected in borosilicate disposable glass tubes containing 100 µl 1 M Tris, pH 7.0, in ~10 fractions, and those fraction with significant absorbance are pooled. The pH of the pooled fractions is neutralized, if necessary, and the sample concentrated to ~0.5 ml using centricon 30 disposable microconcentrators. The sample is dialyzed against 1% N-octylglucoside/buffer. The class II preparations are analyzed using SDS PAGE and coomassie staining, and protein concentration determined using the BCA assay (Pierce) with BSA standards. All of the foregoing steps are performed at 4°C unless otherwise indicated. Columns must be pre-eluted. Depending on the monoclonal antibody and the class II molecule used, the class II molecule(s) may be eluted with lower pH (i.e., pH 10.7). Representative anti-(MHC class II) antibodies are listed in the American Type Culture Collection Catalogue, 7th ed., and include, for example, 14-4-4 for I-E^d and I-E^k, MKD6 for I-A^d, and 10-2-16 for I-A^k.

A mouse of a first strain is given a tolerogenic regimen of injections of the MHC class I antigens from a mouse of a second strain. The first mouse is then tested for the ability to develop cytotoxic T cells (CTL) against the class I MHC antigens expressed by the second strain. CTL responses are typically mainly to class I MHC. Detailed methodology for CTL experiments (also referred to as "cell-mediated cytolysis" or CMC) is given by Hudson et al., in Practical Immunology, 3rd edition, Blackwell, p. 160 (1989), which is incorporated herein by reference.

A similar experiment to determine the ability of soluble class II MHC antigens to induce tolerance involves an increasing dose tolerogenic regimen of administrations of class II MHC molecules in a manner similar to that used for class I molecules. Class II MHC molecules from the second strain mouse are injected in an increasing dose tolerogenic regimen into a mouse of the first strain. Cells obtained from the injected first strain mouse are assayed for a diminished mixed lymphocyte reaction (MLR) against cells from the second strain. This assay reflects the fact that an MLR response is typically mainly to class II MHC. MLR assays are performed according to Meo, in Immunological Methods, Lefkovits and Pernis, eds., vol. 1, p. 227, and as in Hudson et al, supra.

To determine preferred increasing dose tolerogenic regimens, the number and interval of administrations, doses, method of administration, etc. for a particular MHC molecule or molecules to prevent rejection of a particular type of graft, multiple intravenous or intraperitoneal (preferably intravenous) injections, e.g., up to 10 immunizations at intervals of 3 days to 8 weeks, are performed in the range of 100pg to 10 μg per injection per mouse, with the preferred dosage being in the range of 1ng to 1 μg. A preferred regimen includes increasing doses. For example, this involves a series of injections beginning at a dose in the 100pg to 10ng range, and increasing by factors of 3 to 10, for up to 20 injections given at intervals in the range of 3 days to 8 weeks.

To assess the extent of immunological tolerance of a mouse of the first strain that has received an increasing dose regimen of injections of MHC molecules from a mouse of a second strain, skin grafts from naive mice of both the first and second strains and a third strain are applied to representative tolerized mice as well as naive mice, where the first, second and third strains have different MHC haplotypes from each other. The grafting involves the recipients being anaesthetized with sodium pentobarbital given i.p. at a dose of 0.06 mg per gram of body weight. Each mouse receives a

full thickness graft of an allogeneic donor skin and a control graft of syngeneic skin. The tolerized mice retain the second strain grafts longer than do naive mice, while the rate of rejection of skin grafts from mice of the third strain is similar to the naive (untolerized) mice.

EXAMPLE 2

This Example illustrates the administration of the increasing tolerogenic regimen of a combination of an MHC class I antigen(s) and an MHC class II antigen(s), in the inhibition of the development of disease by developing tolerance to one or the other or both substances.

A. Transplantation Tolerance

Transplantation tolerance in an intended graft recipient is induced using an increasing tolerogenic regimen of effective amounts of the foreign polymorphic class I MHC and class II MHC antigens expressed by the graft donor that are not expressed by the recipient. Due to the stimulation of the subset of CD8 cells that recognize both foreign class I MHC and the idiotypes of stimulated CD8 cells, the amount of antigen needed is smaller than in the case of Example 1, and the duration of the injections needed is shorter.

The MHC class I and class II antigens are prepared as described in Example 1. An increasing dose regimen beginning at 0.1 ng/kg to 1 μg/kg of the TCR4-binding substance(s) and 0.1 ng/kg up to 1 μg/kg of the TCR8-binding substance, increasing by a factor of 2 to 10 for one to ten injections at intervals of from 3 days to 10 weeks (preferably one to six weeks) are given over a period. The transplant is performed thereafter, with a boost of the antigens being given one week to one month immediately prior to the transplant to reinforce the suppressed state. The size of the boost is the same as the final dose of the increasing dose regimen, or can

be an order of magnitude less. Comparisons between the use of a single MHC antigen and the combination of class I and class II antigen in inducing tolerance as defined by CTL assays and MLR assays demonstrate the effectiveness of the combination therapy and is confirmed by prolongation of graft survival (e.g. skin graft).

B. Tolerance to Autoimmune Diseases

The combination of class I and class II MHC molecules in preventing autoimmunity can be demonstrated in established animal systems of autoimmune disease, including the NOD mouse model for diabetes, the MRL-lpr/lpr model for systemic lupus erythematosus, and the experimental autoimmune encephalomyelitis (EAE) model for multiple sclerosis.

To demonstrate prevention of autoimmune type diabetes, female NOD mice are injected with I-E antigen together with allogeneic class I MHC molecules prior to the age when diabetes typically develops, i.e., about 3 months, with immunization regimens as given in Example 2, to determine optimum protection against development of diabetes.

To demonstrate the amelioration of symptoms of systemic lupus erythematosus the MRL-lpr/lpr mouse model is used. MRL-lpr/lpr mice make anti-gp120 antibodies (gp120 of HIV), and a change in the T cell repertoire that suppresses the production of these antibodies can be used to suppress pathogenesis. As gp120 and p24 antigens of HIV have been described as MHC-class II mimicking substances (Ziegler and Stites, Clin. Immunol. and Immunopath. 41:305-313 (1986); Andrieu et al. AIDS Research 2:163-174 (1986); Hoffmann et al., Proc. Nat. Acad. Sci. (USA), 88:3060-3064 (1991); Kion and Hoffmann, Science, 235:1138-1140 (1991)), they are preferentially TCR4-binding substances. A combination of allogeneic MHC class I antigen and gp120 is given in an increasing dose regimen according to the immunization schedule described in Example 2. Suppression of the production of anti-gp120 antibodies is determined, conveniently using ELISA assays with recombinant gp120 antigen, and pathogenesis is

more effectively inhibited than if only low doses of gp120 are injected.

Experimental autoimmune encephalomyelitis (EAE), a model of the human disease multiple sclerosis, is a disease that can be induced in susceptible strains of mice and rats by injecting myelin basic protein together with an adjuvant (Arnon, Immunol. Rev. 55:3 (1981); Acha-Orbea et al., Ann. Rev. Immunol. 7:371 (1989)). T cells that are specific for myelin basic protein have been used to vaccinate against EAE (Cohen, J. Int. Med. 230:471-477 (1991)). According to the present invention, an increasing dose regimen of myelin basic protein and class I MHC is injected to change a subject that is susceptible to disease into one that is not susceptible. Since myelin basic protein (MBP) is normally present as a self protein, a relatively large injection of MBP may be needed to perturb the immune system enough to induce a change in the T cell repertoire, involving T cells that recognize MBP, to a more stably suppressed state. Inhibition of encephalomyelitis in immunized animals evidences that tolerance is induced (or stabilized) to MBP.

The invention can also be used for the treatment of autoimmune disease after onset of disease. For example, EAE can be treated with initially small, then increasing doses of MBP together with class I MHC antigen, with an immunization regimen as specified in Example 2. This induces the immune system to switch to a suppressed state for that antigen. Antibodies specific for denatured collagen, DNA and RNP (ribonuclear protein) occur in lupus and several other autoimmune diseases. Initially small, then increasing amounts of one or more of these substances together with small and increasing amounts of allogeneic class I MHC will switch off the undesired autoimmune responses to collagen, DNA and/or RNP according to the antigenic substance which is administered.

EXAMPLE 3

This Example demonstrates the administration of subimmunogenic doses of allogeneic class I MHC antigens and an allergen in therapeutic methods to stabilize an individual's immune reaction to the allergen and desensitization of IgE response to the allergen.

Desensitization is established in an animal model, for example a rat, mouse or guinea pig model. An animal is made allergic to a substance (e.g., a protein such as ovalbumin (OA), or a haptenated protein such as BPO-BGG, DNP-BGG or DNP-OA, where BPO is benzylpenicilloyl, DNP is dinitrophenyl and BGG is bovine gamma globulin (see Levine and Vaz, Int. Arch. Allergy, 39:156-171 (1970) and Lee and Sehon, J. Immunol. 114:829-836 (1975), both of which are incorporated herein by reference). In the mouse, intraperitoneal injections of just 1 μg of a DNP-OA conjugate in the presence of 1 mg of freshly prepared aluminum hydroxide in 0.5 ml of saline induces a significant IgE producing cell response, as measured in the heterologous adoptive cutaneous anaphylaxis assay described by Kind and Macedo-Sobrinho (J. Immunol. 111:638 (1973), incorporated herein by reference). Desensitization occurs most effectively by giving injections of increasing doses of the allergen together with increasing doses of class I MHC antigens in an immunization schedule as described in Example 2 above. A more rapid and effective desensitization is achieved than using conventional desensitization protocols.

In one study, C3H mice were sensitized with antigen (hen egg lysozyme, HEL) to produce an IgE response. The allergic sensitivity of the animals to the allergen was then reduced by treatment with increasing dosages of antigen plus soluble allogeneic MHC-I.

C3H mice were sensitized by two subcutaneous injections of 30 μg of HEL at three week intervals. One group was untreated. Mice were given i.v. injections of HEL in PBS at escalating dosages (pg-ng range), or escalating doses of

antigen (HEL) plus H-2K^b (MHC-1). Injections occurred weekly, over a 6 week period. The dose for group 3 began at 0.9 pg MHC-1 and 3pg HEL, and increased by a factor of 10 each week to final doses of 90ng and 300ng respectively. Group 4 received the same series of injections of HEL without the MHC-1. Control mice received to treatment. On week 12 all mice were bled to determine the histamine response. The decrease observed in the histamine level of the mice treated with escalating levels of antigen was not statistically significant. The mean histamine response of mice that received the combination of MHC-1 and antigen was less than half that of control mice that were not desensitized.

Plasma histamine in sensitized C3H mice after treatment with MHC-1 plus antigen

Group:	1	2	3	4
Treatment:	sensitized not treated	not sensi. not treated	sensitized MHC-1/HEL	sensitized HEL only
mean ng/ml (8EM)	8544 (1340)	211 (27)	4161 (628)	5519 (1419)
difference from group 1	-	-	P=0.055	P=0.11

Notes:
a) Mean and SEM titre per group, calculated on log transformed data.
b) Comparisons were by one-tailed Students t, on logarithmic data, 95% confidence (0.05 is significant).

EXAMPLE 4

This Example demonstrates that xenogeneic MHC molecules can be administered in subimmunogenic amounts for inhibiting the development of AIDS.

A combination of xenogeneic class I MHC and class II MHC molecules from a species not susceptible to simian AIDS, such as the African green monkey, is injected in

subimmunogenic doses into animals of a species that is susceptible to simian immunodeficiency virus (SIV) induced disease (e.g. rhesus macaque monkey). Injections are given as specified in Example 2. The experimental group of monkeys is then challenged with a dose of SIV that reliably causes simian AIDS in control animals, and monitored for the development of symptoms of simian AIDS. Animals receiving the increasing dose regimen of class I and class II MHC molecules demonstrate an inhibited development of symptoms of immunodeficiency disease.

EXAMPLE 5

This Example describes methods to identify substances that bind preferentially TCR4 or TCR8 (e.g., anti-I-J antibodies, and the 1F7 antibody described in Wang et al., Eur. J. Immunol. 22: 1749-1755 (1992), incorporated herein by reference), in addition to MHC class II and MHC class I molecules, which can be used as positive controls to develop and standardize the methods.

A T cell preparation is depleted of CD4 or CD8 cells using anti-CD4 or anti-CD8 reagents, either with complement, or by the application of an immunoabsorbant method. Differences in binding of a fluorescence-dye labeled conjugate of the substance being screened to CD4 and CD8 T cells are determined, conveniently using flow cytometry.

Alternatively, differences are determined between the in vitro proliferative responses, in the presence of added IL-2, of CD4 and CD8 T cells to either the native substance or to a form of the substance that has enhanced ability to cross-link receptors, e.g., the substance bound to a plate or beads. The substance may also be incubated with the CD4 or CD8 T cells, then specific antibodies to the substance added to enhance receptor cross-linking.

EXAMPLE 6

This Example demonstrates that self MHC molecules can be administered in sub-immunogenic doses to both prevent and provide therapy for an autoimmune disease.

The MRL-lpr/lpr mouse, which spontaneously develops fatal autoimmunity, has the H-2^k MHC haplotype. Protection from disease occurs most effectively by giving injections of increasing doses of MHC class II molecules of the H-2^k haplotype, namely I-A^k and/or I-E^k, in combination with allogeneic class I MHC molecules. The self class II MHC molecules may be aggregated or covalently linked, or they may be covalently coupled to the class I MHC molecules. The MHC molecules are injected into three week old MRL-lpr/lpr mice beginning at very low doses, as specified in Example 2. Animals receiving an increasing dose regimen demonstrate an inhibited development of autoimmune disease.

Older animals that already have autoimmune disease (from about 3 months of age) that are treated in the same way demonstrate a remission of the various symptoms, including lymphadenopathy and the production of autoantibodies, that are typically associated with autoimmunity in these animals.

EXAMPLE 7

C3H mice were given six escalating dosages (pg-μg range) of H-$2K^b$ molecules (MHC-1), namely doses of 90pg, 900pg, 9ng, 90ng, 900ng and 9μg. Injections occurred weekly, over a 6 week period. Two control groups received no treatment. On weeks 7 and 10 all mice (except one of the control groups) were given 30 μg of antigen (hen egg lysozyme, HEL) by subcutaneous injections, in a protocol known to induce IgE and IgG1 antibody and allergic hypersensitivity. On week 13 all mice were bled to determine antibody levels and challenged with an i.p. injection of HEL. Plasma was

collected 8-10 min. after challenge to determine the histamine response.

Mice treated with MHC-1 produced high levels of IgG1 antibody (11-fold higher than untreated controls). This result was statistically significant (greater than 95% confidence). 100% of the MHC-1 treated mice responded to the antigen, in contrast to only 67% of untreated controls. The average ratio of histamine related in response to antigen to the level of antigen-specific IgG1 was decreased by a factor of greater than 7 in the treated mice.

Anti-HEL IgG1 Antibody, Histamine in Serum of Mice Treated With MHC-1

Group:	1	2	3
Treatment: Antigen	not treated sensitized	not treated not sensitized	MHC-1 sensitized
mean titer IgG1 (SEM)	760 (340)	<50 (-)	8200 (7400)
IgG1+/total	4/6	0/6	5/5
Compare to Group #1	-	-	P=0.03
histamine ng/ml mean (SEM)	2773 (2239)	107 (31)	5728 (1858)
histamine/IgG1 ratio arbitrary units (SD)	3.8 (4.9)		0.55 (0.38)

a) Mean and SEM titre per group, calculated on log transformed data.
b) IgG1+/total - fraction of mice with detectable IgG1, some mice in Group #1 had no IgG1.
c) Bleeds from all groups post-treatment and before sensitization were all negative for antigen-specific antibody, i.e., no detectable response to MHC-1 during treatment.
d) Comparisons were by Students t, on logarithmic data, $P<0.05$ is significant.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for inhibiting graft rejection in a subject, which comprises

administering to said subject prior to transplantation of said graft a subimmunogenic amount of a composition which comprises MHC class I and class II antigens of the graft to be transplanted and a physiologically acceptable carrier.

2. The method of claim 1, which further comprises administering subsequent additional doses of said MHC antigens to the subject prior to transplantation, in amounts which individually are equal to or greater than a previous dose.

3. The method of claim 1, wherein the graft is an allograft and the MHC antigens are alloantigens.

4. The method of claim 1, wherein the graft is a xenograft and the MHC antigens are xenogeneic antigens from a species which is the source of the graft.

5. The method of claim 1, wherein the MHC class I and MHC class II antigens are covalently linked to each other.

6. A method for inhibiting autoimmune disease in a subject, which comprises

administering to said subject a subimmunogenic amount of a composition which comprises an MHC class II molecule associated with the absence of the autoimmune disease, TCR8 binding antigen, and a physiologically acceptable carrier.

7. The method of claim 6, wherein the TCR8 binding antigen is an MHC class I molecule.

8. The method of claim 7, wherein the MHC class I and class II molecules are allogeneic MHC molecules.

9. The method of claim 7, wherein the MHC class I and class II molecules are xenogeneic MHC molecules from a species which does not suffer from the autoimmune disease.

10. The method of claim 9, wherein the MHC class I and class II molecules are covalently linked to each other.

11. The method of claim 6, wherein the soluble MHC class II molecule and TCR8 binding antigen are covalently linked to each other.

12. The method of claim 6, which further comprises administering subsequent additional doses of said MHC class II molecules and TCR8 binding antigen to the subject susceptible to said autoimmune disease, in amounts which individually are equal to or greater than a previous dose.

13. A method for inhibiting immunodeficiency disease in a subject, which comprises

administering to said subject a subimmunogenic amount of a composition which comprises MHC class I and MHC class II antigens and a physiologically acceptable carrier.

14. The method of claim 13, which further comprises administering subsequent additional doses of said MHC antigens to the subject in amounts which individually are equal to or greater than a previous dose.

15. The method of claim 13, wherein the MHC antigens are alloantigens.

16. The method of claim 13, wherein the MHC antigens are obtained from a xenogeneic species which is not prone to developing said immunodeficiency disease.

17. The method of claim 13, wherein the MHC class I and class II antigens are covalently linked to each other.

18. A method for inducing immunological tolerance to an antigen in a subject, which comprises

administering to the subject a composition which comprises a subimmunogenic amount of the antigen and a subimmunogenic amount of one or more MHC molecules.

19. The method of claim 18, which further comprises administering subsequent additional doses of said MHC molecules to the subject in amounts which individually are equal to or greater than a previous dose and which induce or maintain said tolerance to the antigen.

20. The method of claim 18, wherein the MHC molecule and the antigen are covalently linked to each other.

21. The method of claim 18, wherein the MHC molecule is selected from MHC class I, MHC class II, or a combination of MHC class I and II molecules.

22. The method of claim 18, wherein the MHC molecules are xenogeneic molecules.

23. The method of claim 18, wherein the antigen is one to which the subject is predisposed to suffer an allergic reaction.

24. The method of claim 18, wherein the antigen is a self antigen to which the subject is predisposed to make autoantibodies.

25. A method for inducing suppressor T cells in an individual which comprises

administering to the individual a composition which comprises a subimmunogenic amount of TCR4 and TCR8 binding substance, and a physiologically acceptable carrier.

26. A method for inhibiting autoimmune disease in an individual comprising

administering to said individual a subimmunogenic amount of a composition which comprises MHC class I and MHC class II molecules, wherein at least one of said MHC molecules is from said individual, and a physiologically acceptable carrier.

27. The method of claim 26, wherein said MHC class I and class II molecules are covalently linked.

28. The method of claim 26, wherein each of said MHC class I and class II molecules are obtained from said individual.

29. A method for activating an immune response to an antigen of interest in a subject, which comprises

administering to said subject prior to administration of the antigen a soluble foreign MHC class I molecule or fragment thereof, in a physiologically acceptable carrier.

30. The method of claim 29, wherein the MHC class I molecule or fragment thereof is administered in an initial subimmunogenic amount and in at least one additional dose in amounts which individually are equal to or greater than a previous dose.

31. The method of claim 29, wherein the foreign MHC class I molecule is allogeneic or xenogeneic.

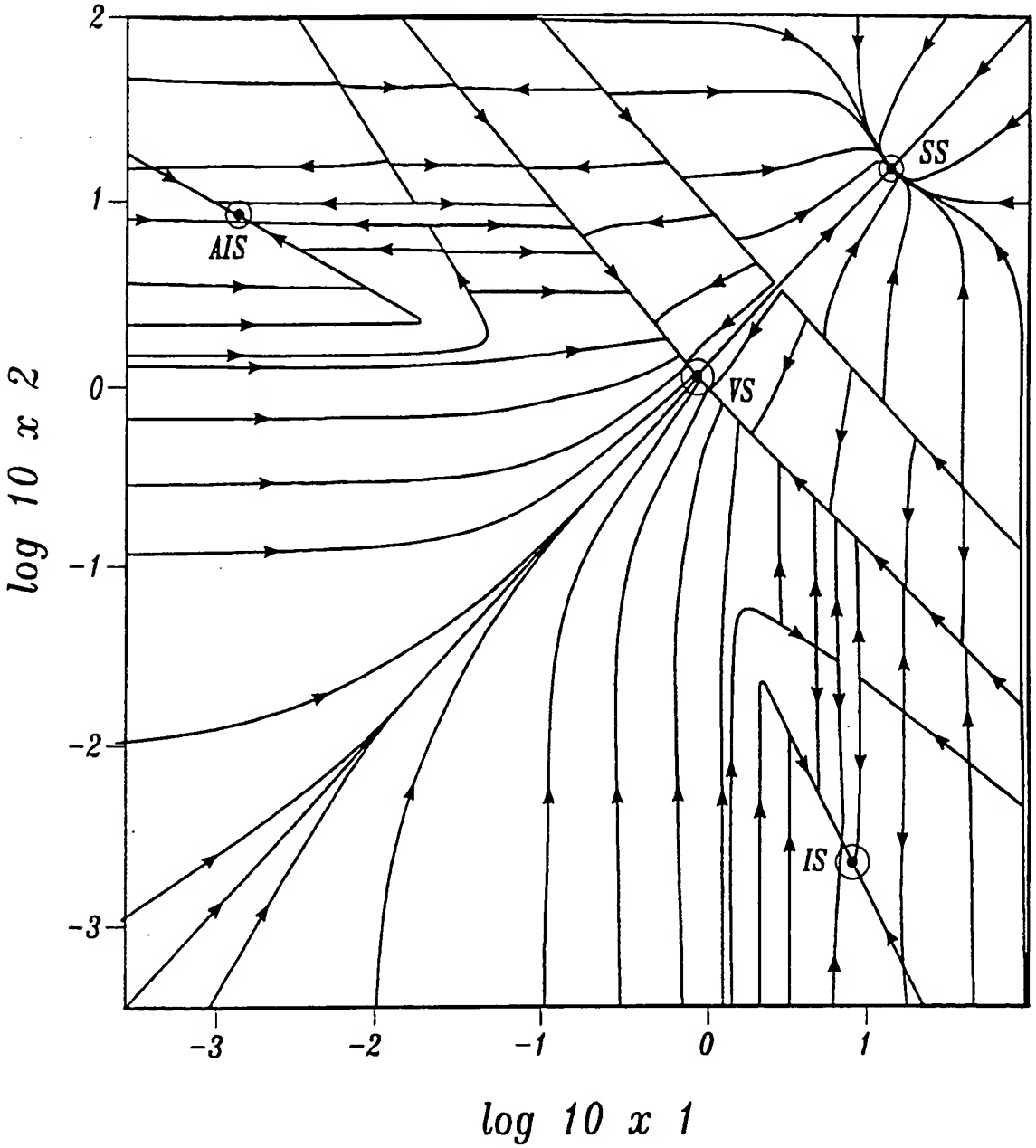
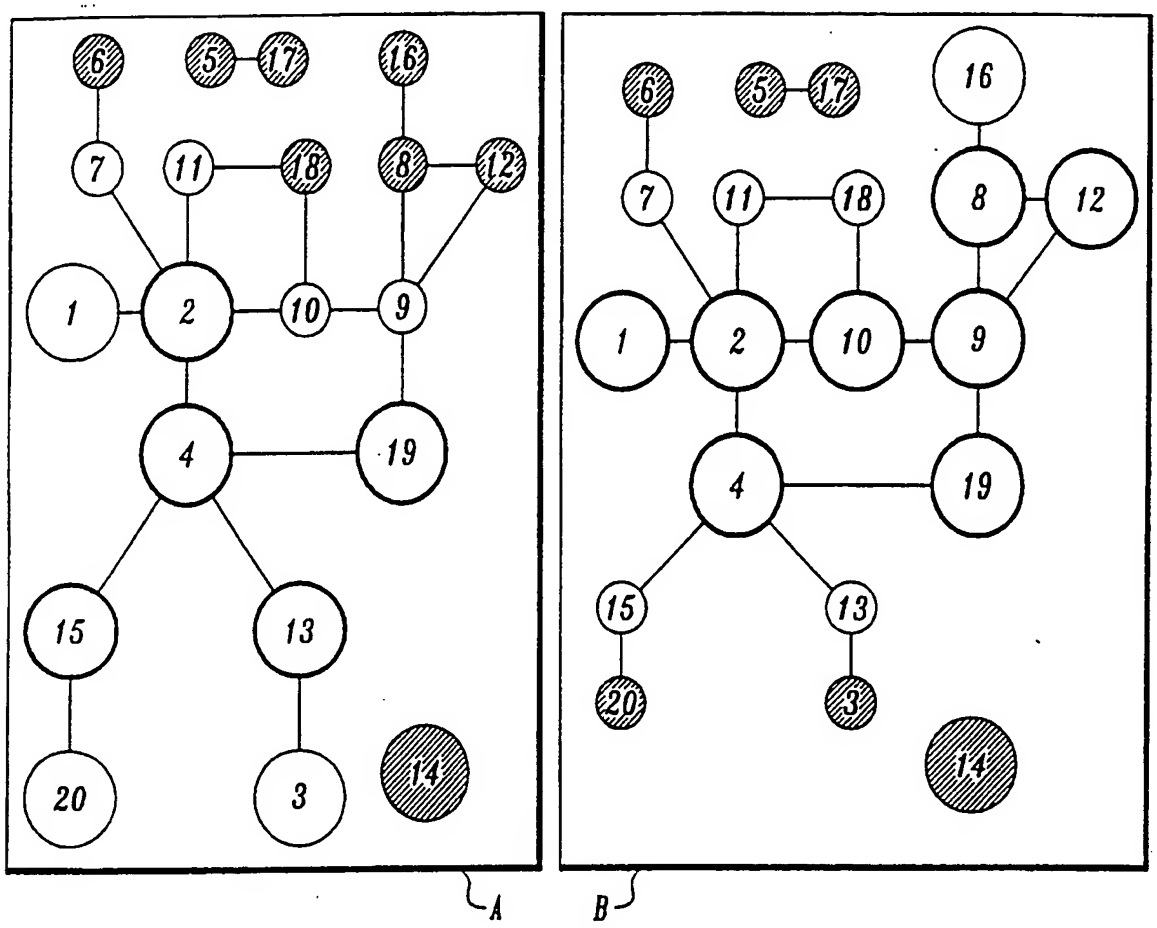


FIG. 1.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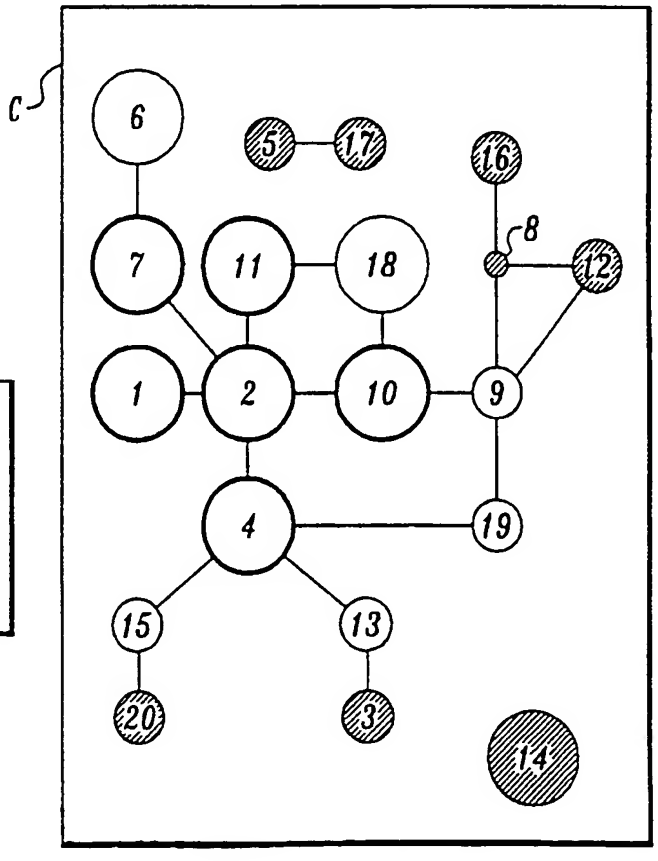
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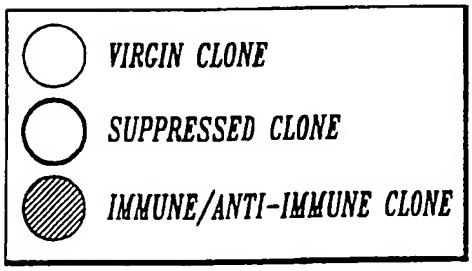
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IMMUNE/ANTI-IMMUNE CLONE

FIG. 2.

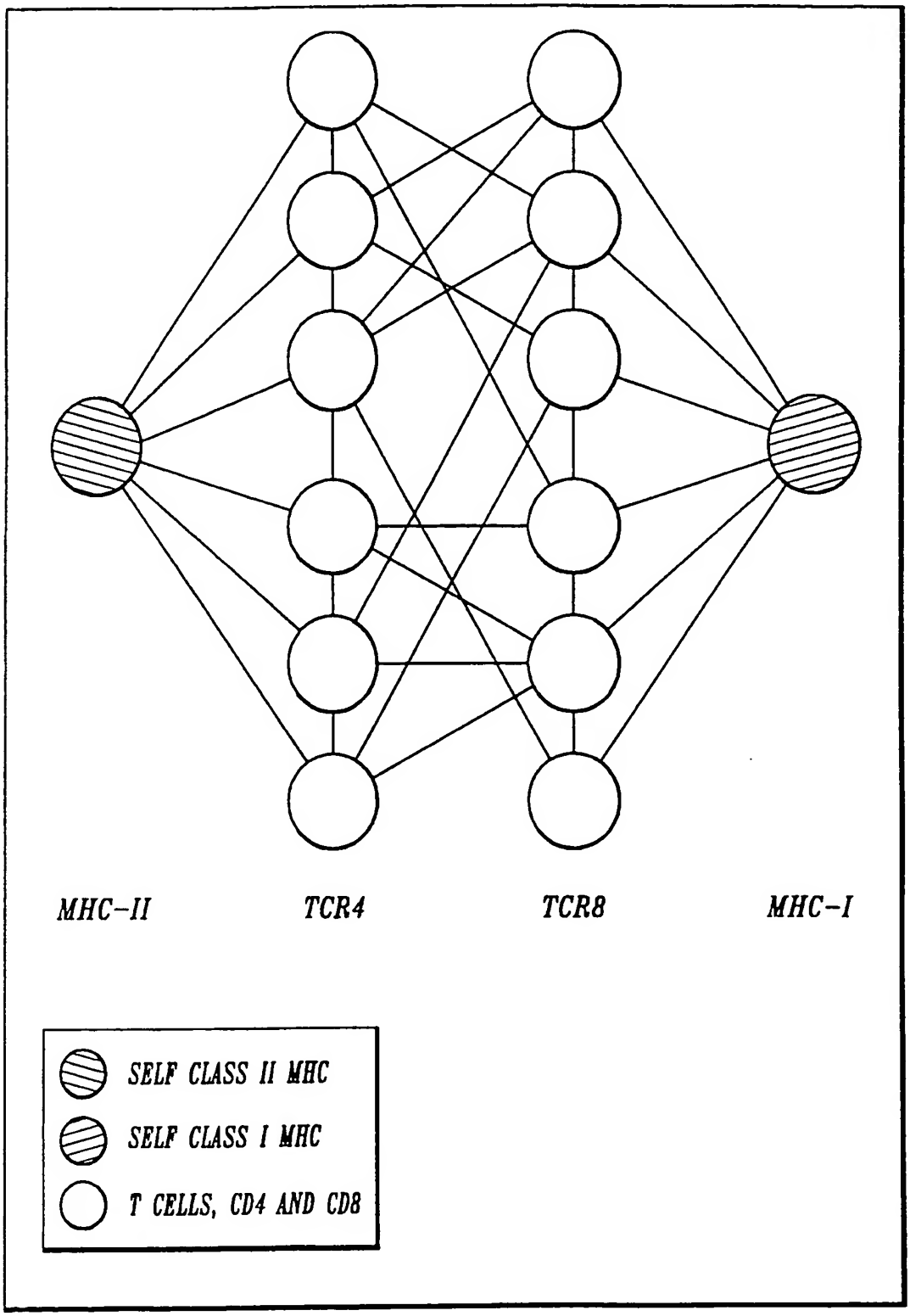
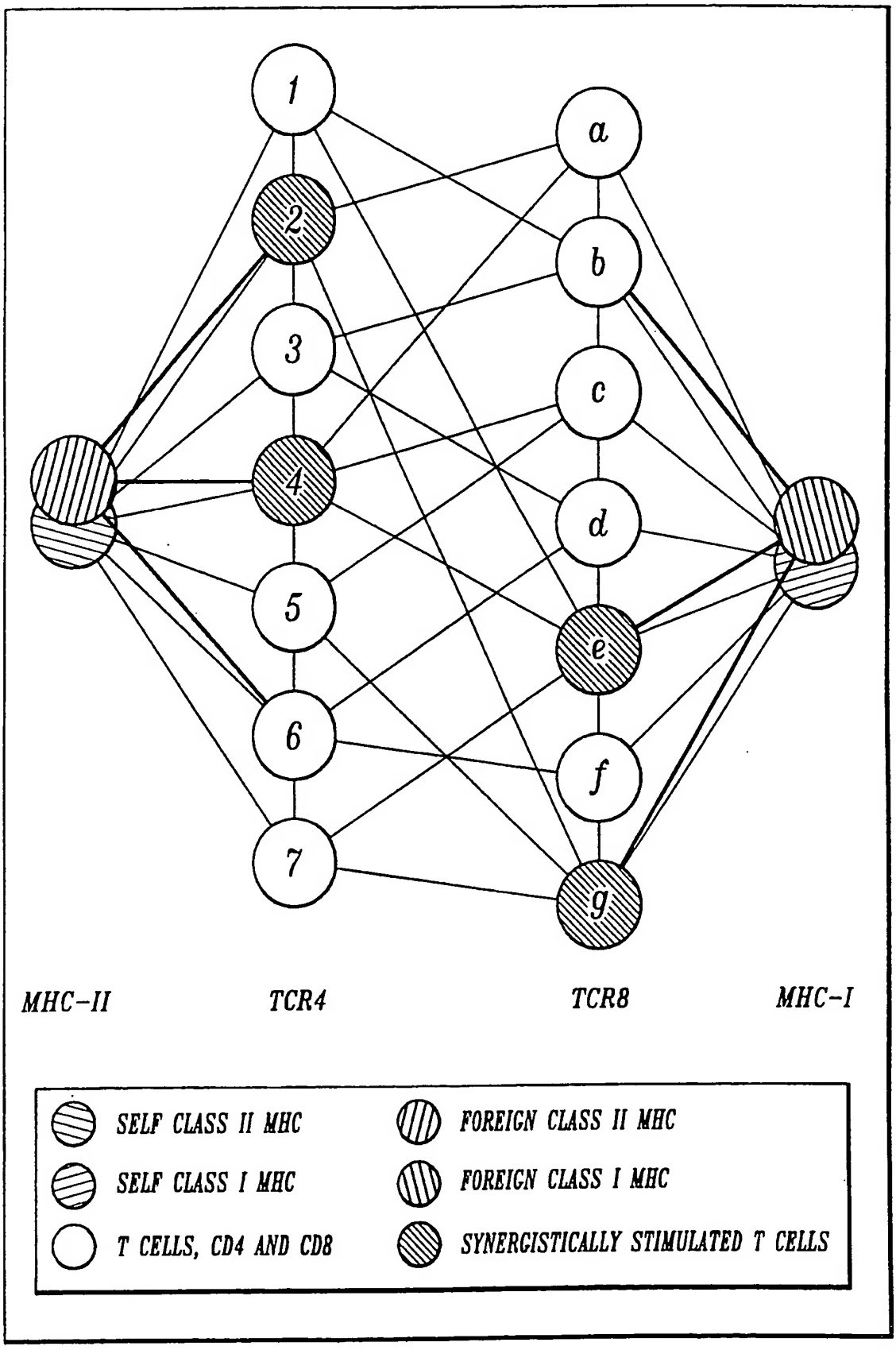

MHC-II
TCR4
TCR8
MHC-I
SELF CLASS II MHC
SELF CLASS I MHC
T CELLS, CD4 AND CD8

FIG. 3.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f
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TCR4
TCR8
MHC-I
SELF CLASS II MHC
SELF CLASS I MHC
T CELLS, CD4 AND CD8
FOREIGN CLASS II MHC
FOREIGN CLASS I MHC
SYNERGISTICALLY STIMULATED T CELLS

FIG. 4.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 94/00083

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 39/00,A 61 K 39/385

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A1, 92/18 150 (ANERGEN, INC.) 29 October 1992 (29.10.92), abstract; claims. --	1-31
A	WO, A1, 88/05 784 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 11 August 1988 (11.08.88), page 3, line 24 - page 12, line 27. ----	1-31

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 June 1994	01. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	SCHNASS e.h.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 94/00083

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-31
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although all claims are directed to a therapeutical method of treatment for human body (Rule 39.1(iv)PCT) the search has been carried out.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/CA 94/00083 SAE 87077

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

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